Endotoxemia Following Experimental Intestinal Strangulation Obstruction in Ponies

J.N. Moore, N.A. White, J.N. Berg, C.M. Trim and H.E. Garner*

ABSTRACT

Experimental small intestinal strangulation obstruction was produced in anesthetized ponies. The limulus amoebocyte lysate test demonstrated the presence of endotoxin in the general circulation 60 and 120 minutes following restoration of mesenteric blood flow. Mucosal degeneration, with loss of villus epithelial cells, was demonstrated coincident with endotoxemia. The findings were consistent with an ischemia-mediated alteration in the intestinal barrier to endotoxin.

RÉSUMÉ

Cette expérience consistait à provoquer une obstruction par strangulation de l'intestin grêle, chez des poneys anesthésiés. L'épreuve du lysat d'amibocytes du limule démontra la présence d'endotoxine, dans la grande circulation, au bout de 60 à 120 minutes après le rétablissment de la circulation sanguine mésentérique. On démontra que la dégénérescence de la muqueuse intestinale et la desquamation épithéliale de ses villosités coïncidaient avec l'endotoxémie.

Les résultats de cette expérience s'avérèrent compatibles avec une altération de la barrière intestinale à l'endroit de l'endotoxine, imputable à l'ischémie.

Intestinal strangulation obstruction (ISO) of the small intestine occurs most frequently in man and the horse. The condition involves interruption of intestinal vascular supply and total occlusion of the intestinal lumen. The prognosis for ISO is poor in both species with mortality rates as high as 35% in human cases (11) and 46.6% in the horse (12). Shock is associated with ISO and is termed septic, hypovolemic, or endotoxic shock (2, 3). Signs include tachycardia, tachypnea, hypotension, hemoconcentration with dehydration, poor capillary refill, neutropenia, and metabolic acidosis (primarily lactic acidosis). Without appropriate supportive therapy and surgical correction of ISO, cardiovascular collapse and death ensue. The circulatory shock associated with ISO in the horse is presumed to be due to endotoxemia. We therefore investigated whether or not circulating endotoxin was present in experimental ISO in ponies and if endotoxemia could be related to mucosal morphology.

Four adult ponies weighing approximately 125 kg were anesthetized with intravenous sodium pentobarbitol. Baseline and subsequent jugular blood samples were obtained by venipuncture through previously surgically prepared skin. A midline celiotomy was performed and the distal jejunum exteriorized. Loose umbilical tape ligatures were placed around the last eight arterial and venous jujunal loops (supplying the terminal 3-4 meters of jejunum). Similarly, loose umbilical tape ligatures were placed around the ileal artery and around the intestine at the level of the proximal and distal vascular ligatures. A full thickness jejunal biopsy (control) was obtained with a 7 mm biopsy punch, and the site oversewn. Care was taken to prevent spillage of intestinal contents or subsequent leakage. Subsequent biopsies were obtained in a similar manner.

After the control samples were obtained, the preplaced ligatures were tightened in two ponies creating ISO. The exteriorized intestine was returned to the abdomen and the incision temporarily closed with towel clamps. Two ponies were sham operated (ligatures not tightened) with biopsies and blood samples taken. The duration of ISO was either 50 or 180 minutes. All ponies were monitored for 120 minutes following release of ISO and then euthanized. Intestinal biopsies were placed in buffered formalin, sectioned, and stained with hemtoxylin and eosin for histopathological grading of villus mucosal lesions (13).

Blood samples were assayed for endotoxin using limulus amoebocyte lysate (LAL) procedure (1).

*Department of Veterinary Medicine and Surgery (Moore, Trim and Garner), Department of Veterinary Microbiology (Berg), College of Veterinary Medicine, University of Missouri-Columbia, Columbia, Missouri 65211 and Department of Large Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602 (White). Present address of Drs. Moore and Trim: Department of Large Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602.

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TABLE I. Results of Limulus Amoebocyte Lysate Analyses of Jugular Blood for the Detection of Endotoxin Prior to and Following Intestinal Strangulation Obstruction

Pony	Duration of ISO	Base	Time During Obstruction 30	60	90	120	180	Time Following Release 10	30	60	120
1	Sham	Neg	—	Neg	—	Neg	Neg	—	—	Neg	Neg
2	Sham	Neg	—	Neg	—	Neg	Neg	—	—	Neg	Neg
6	50 min	Neg	Neg	—	—	—	—	Neg	Neg	Pos	Pos
5	180 min	Neg	—	Neg	—	Neg	Neg	Neg	Neg	Pos	Pos

Neg = Negative
Pos = Positive
— = Not performed
ISO = Intestinal strangulation obstruction

Three milliliters of blood were collected into endotoxin-free heparinized tubes. Plasma was collected, diluted 1:3 with endotoxin-free water and heated at 100°C in a heat block. Dilution and heating inactivate inhibitors of the LAL that are present in plasma. The samples were agitated with a vortex mixer, and 25 μL of the plasma withdrawn and added to a tube containing 25 μL of LAL.[1] This tube was incubated for one hour in a water bath. The formation of a solid clot in the tube was read as a positive test. Controls used included positive (1 ng endotoxin/mL blood) and negative blood samples, and positive (0.1 ng endotoxin/mL water) and negative water samples. The lysate used had a stated sensitivity of 0.06 ng/mL and has been shown to have a sensitivity 0.1 ng/mL blood when known amounts of endotoxin are added to horse blood.

Results of limulus lysate assays for circulating endotoxin are presented in Table I. Endotoxin was not detected at any time in the sham operated ponies. Endotoxin was present in blood samples 60 and 120 minutes after ligature release in ponies 5 and 6. Mucosal alterations (graded 0-V) are presented for each intestinal biopsy in Table II (13).

Endotoxin is the lipopolysaccharide component of the cell wall of gram negative bacteria (7). Free endotoxin usually arises from death and lysis of the bacterium although it may also be present during rapid growth phases. Under normal conditions when intestinal mucosal cells are viable and functioning, free endotoxin will largely be confined to the intestinal lumen. The small amount of endotoxin normally absorbed from the intestine is rapidly removed from the portal blood by hepatic Kupffer cells (6, 8, 10). Disruption of intestinal vascular supply, however, alters the functional capacity of mucosal cells and the barrier to movement of bacteria and endotoxins is lost. Under such circumstances, the majority of endotoxin passes transmurally into the peritoneal cavity (9) or escapes via the lymphatics (4). Entry of endotoxin into the systemic circulation by either portal, lymphatic or peritoneal route may result in endotoxemia.

Biological documentation of endotoxemia has been fraught with difficulties. An extract of the amoebocytes of the horseshoe crab, *Limulus polyphemus*, has been found to react with endotoxin forming a gelatinous precipitate. This reaction has formed the basis of the limulus lysate test for endotoxin (7). The test does have specific limitations and is best used as a qualitative means of detecting endotoxin in minute amounts. Recent modifications of the test have resulted in its adaptation to the detection of endotoxin in the blood of domestic animals (1).

In the present investigation, endotoxemia was not demonstrated during the period of intestinal strangulation obstruction nor was it detected ten or 30 minutes after ligature removal. Endotoxin was consistently detected, however, in the systemic circulation 60 and 120 minutes following restoration of mesenteric blood flow in ponies 5 and 6 (Table I) and coincided with marked alterations in mucosal integrity (Table II). These findings together confirm previous clinical impressions that disruption of the mucosal barrier to

TABLE II. Small Intestinal Mucosal Lesions in Biopsies Obtained Prior to, During and Following Intestinal Strangulation Obstruction (13)

Pony	Duration of ISO	Base	Time During Obstruction 30	60	90	120	180	Time Following Release 60	120
1	Sham	0	—	0	—	0	0	0	0
2	Sham	0	—	0	—	0	0	0	0
6	50 min	0	I	—	—	—	—	II	III
5	180 min	0	—	II	—	III	III-IV	IV-V	IV-V

ISO = Intestinal strangulation obstruction
Grade 0 — Normal mucosa
Grade I — Slight separation of epithelial cells from the lamina propria
Grade II — Loss of epithelial cells from the tip of the villus and minimal hemorrhage into the lamina propria
Grade III — Extension of the subepithelial space down the sides of the villus to expose the upper 1/3-1/2 of the lamina propria
Grade IV — Complete separation of epithelium from lamina propria to the villus base with marked lamina propria hemorrhage and edema in the submucosa
Grade V — Loss of villus architecture. Early necrosis has occurred in the neck cells of the crypts

[1]Associates of Cape Cod, Inc., Woods Hole, Maine.

endotoxin occurs following ISO in the horse and that restoration of intestinal perfusion does not necessarily arrest morphological change in the affected bowel.

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REFERENCES

1. **BERG, J.N., L. RIEGLE** and **C.A. NAUSLEY.** Heat extraction of animal plasma in preparation for endotoxin testing with the limulus amoebocyte lysate test. Am. J. vet. Res. 40: 1048-1049. 1979.
2. **BURROWS, G.E.** and **J. CANNON.** Endotoxemia induced by rapid intravenous injection of *Escherichia coli* in anesthetized ponies. Am. J. vet. Res. 31: 1967. 1970.
3. **CARROLL, E.J., O.W. SCHALM** and **J.D. WHEAT.** Endotoxemia in a horse. J. Am. vet. med. Ass. 146: 1300-1303. 1965.
4. **GANS, H.** and **K. MATSUMOTO.** The escape of endotoxin from the intestine. Surg. Gynecol. Obstet. 139: 395-402. 1974.
5. **HINSHAW, L.B., B. BENJAMIN** and **D.D. HOLMES.** Responses of the baboon to live *Escherichia coli* organisms and endotoxin. Surg. Gynecol. Obstet. 145: 1-11. 1977.
6. **JACOB, A.I., P.K. GOLDBERG, N. BLOOM, G.A. DEGENSHEIN** and **P.J. KOZINN.** Endotoxin and bacteria in portal blood. Gastroenterology 72: 1268-1270. 1977.
7. **LEVIN, J., T.E. POORE** and **N.S. YOUNG.** Gram-negative sepsis: detection of endotoxemia with the limulus test. Ann. Intern. Med. 76: 1-7. 1972.
8. **MORI, K., K. MATSUMOTO** and **H. GANS.** On the *in vivo* clearance and detoxification of endotoxin by lung and liver. Anim. Surg. 177: 159. 1973.
9. **NOLAN, J.P.** and **M.V. ALI.** Effect of cholestyramine on endotoxin toxicity and absorption. Am. J. Dig. Dis. 17: 161-166. 1972.
10. **RAVIN, H.A., D. ROWLEY, C. JENKINS** and **J. FINE.** On the absorption of bacterial endotoxin from the gastrointestinal tract of the normal and shocked animal. J. exp. Med. 112: 783-792. 1960.
11. **SHATILA, A.H., B.E. CHAMBERLAIN** and **W.R. WEBB.** Current status of diagnosis and management of strangulation obstruction of the small bowel. Am. J. Surg. 132: 299-303. 1976.
12. **TENNANT, B.C., J.D. WHEAT** and **D.M. MEAGHER.** Observations on the causes and incidence of acute intestinal obstruction in the horse. Proc. Am. Ass. Equine Practnrs. pp. 257-271. 1972.
13. **WHITE, N.A., J.N. MOORE** and **C.M. TRIM.** Mucosal alterations in experimentally induced small intestinal strangulation obstruction in ponies. Am. J. vet. Res. 41: 193-198. 1980.

ADDENDUM

The authors of "Factors Associated with Morbidity and Mortality in Feedlot Calves: The Bruce County Beef Project, Year Two" (Can. J. comp. Med. 45: 103-112. 1981) and "The Interpretation of Antimicrobial Susceptibility Patterns" (Can. J. comp. Med. 45: 199-202. 1981) wish to acknowledge the financial support of the Ontario Ministry of Agriculture and Food, The Ontario Cattlemen's Association and the Bruce County Cattlemen's Association. We apologize for not citing this support previously.